LIFE CYCLE OF *NASSULA ORNATA* AND *NASSULA ELEGANS:* ARE THESE SPECIES VALID?

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I. Description.

Nassula is a ciliated protozoan living in fresh water where there is an abundance of *Oscillatoria* and not too much sunlight.

Laboratory cultures of *Nassula* for these experiments were maintained by placing them in ordinary square watch glasses with spring water and fresh *Oscillatoria*, and these in turn, placed in large covered glass vessels containing a small quantity of water. The cultures were kept in subdued light as direct sunlight was found to be fatal. All work on *Nassula* was done with pure lines started from one individual and maintained for nearly a year.

The genus *Nassula* was first described by Eherenberg about 1830. His description includes the species *Nassula ornata* and *Nassula elegans*, the main difference between the two being color, shape, and size. *N. ornata* he describes as being ovate or cylindrical, dark green or violet in color and about 200–240 micra in length. *N. elegans*, he gives as varying in color from red to transparent, elongated, and about 160 micra in length (Eyferth, 1900).

However there is great confusion in the classification of *Nassula*. Pritchard (61), describes *N. ornata* and *N. viridis* as the same species while Eyferth (00), describes *N. ornata* and *N. aurea* as the same. The species *N. elegans* as described by Pritchard is exactly opposite to the description given by Cohn (53), for the same species.

Not only the species are confused, but also the genus, as is shown by the following: "Stein hints it as probable that this species (*N. aurea*) and *N. viridis*, *Chilodon aureas* and *Ch. ornatus* are merely different stages of the same animal" (Pritchard, 61).

The entire body of *Nassula* is covered with cilia appearing in rows, approximately longitudinally disposed, over its surface.

The nuclear complex is peculiar in that it is not differentiated into micro- and macro-nuclei but is very similar to the nucleus of *Amœba proteus*. This statement does not agree with Cohn (53), who says of *N. elegans*, "Nucleus elliptic, with nucleolus lodged at one end." And Pritchard (61), with *N. elegans* in mind, thinks the "nucleus is stoutly clavate, and terminated by a small oblong nucleolus at its narrower extremity." The nucleus does not occupy a fixed position but is moved around by the streaming of the protoplasm.

The mouth opens into a pharyngeal apparatus composed of about twenty-five pharyngeal rods arranged in the form of a truncated cone, with the base anteriorly directed. This pharyngeal apparatus is described by all of the above mentioned authors for all species with the exception of Cohn (53), who states that there is no such apparatus in *N. elegans*. About one third of the way posterior to the base of this basket-like cone appears to be a "band of refractive protoplasm," as described by Eherenberg (00), for *N. elegans*. Nothing was said of this ring in *N. ornata*. However, I have been able to demonstrate this ring in prepared material of both species. Eherenberg probably overlooked it in living specimens of *N. ornata* because of its being obscured by the food vacuoles. This mechanism seems to serve as a support to the pharyngeal rods and also aids in drawing *Oscillatoria* filaments through the pharynx into the body of the animal.

Food vacuoles are numerous and vary in number and color with the amount of food injested and the stage of digestion of this food. A freshly fed *Nassula* contains so many food vacuoles of such dark color that the other morphological details cannot be made out with certainty. Just after injestion of food the vacuoles are brown or dark green due to the color of the *Oscillatoria*, but as digestion proceeds they are changed to a shade of purple, then pink and finally faint straw color. All these stages may be encountered at one time in the same individual.

The contractile vacuole, of which there is but one, is peculiar. It is stationary and is the point toward which the streaming of the protoplasm is directed. As metabolism ensues minute vacuoles of clear fluid are formed throughout the protoplasm. With the streaming of the protoplasm these vacuoles are brought into close contact with the point at which the contractile vacuole is formed. As the minute vacuoles come together they fuse to form a larger vacuole, which, when its maximum size is reached, is discharged. Thus there can always be seen a larger contractile vacuole with smaller vacuoles of various sizes surrounding it, while others are drifting through the cytoplasm toward it. At the point of excretion is situated a wedge-shaped pore connecting the contractile vacuole with the outside of the animal, and through which the waste material passes. Waste material is discharged at the rate of about eighty seconds.

Nassula thrives only on *Oscillatoria*. Although I have observed *Nassula* containing a few desmids, I have never been able to maintain a culture using desmids as food. *Nassula* divides by transverse fission. The nuclear conditions during division have not yet been completely worked out but work is being done at the present time on this subject. Binary fission is found to occur more frequently in older cultures.

2. No Warrant for Two Species.

The amount of food present plays a great part in the life history of *Nassula*. As long as there is an abundance of *Oscillatoria* the animals, which were classified as *N. ornata* by Eherenberg, live and multiply in the normal way and continue to present the color, size and contour of *N. ornata*. But as the food supply becomes exhausted they undergo noticeable changes in these features. As stated above *N. ornata* is normally ovate or cylindrical, brown or dark green in color and about 200 micra long, but as the food supply diminishes the animal becomes very much smaller and is decidedly longer than it is wide. The color of the food vacuoles changes to a faint red. In this condition it meets all descriptions of *N. elegans*. If, when this stage is reached, *Oscillatoria* is fed to the animals, they immediately attach themselves to the filaments to feed. Several hours later they will be seen to contain numerous food vacuoles of very dark color and their normal size is again reached, now meeting Eherenberg's description of *N. ornata*. Thus, by withholding food, *N. ornata* may be made to assume the characteristics of *N. elegans*. Age of the culture also, as over against lack of food, plays a small part in the converting of *N. ornata* into the so-called *N. elegans*. Older cultures of *N. ornata* change a short while before those in which the water is fresher. This will be seen by referring to Table I. Hence we find that *N. ornata* and *N. elegans* are but metabolic phases of a single species based upon nutritional and toxic conditions.

3. Encystment.

As stated above, after food has been withheld from *N. ornata* the animals develop the characteristics of *N. elegans*. If now, food is again added to these specimens a reversal will occur and the animals will again appear as *N. ornata*. This may be carried on indefinitely making alternate sequences of the forms so long as food is withheld and added at the proper time and in the proper sequences. However, if no food is added, the animals eventually become much lighter in color than even *N. elegans*, becoming almost transparent. Then encystment takes place. Thus it happens that they enter the cysts as *N. elegans*, *N. ornata* has not been observed to encyst.

Age of the culture aids in encystment but is not the basic cause as will be seen from the following specific experiment:

Four cultures were started as follows: All the *Nassulas* being taken from the same culture which was a sub-culture of the original pure line.

Culture No. 1.—Water from a culture of *Nassulas* several weeks old was filtered and placed in a watch glass with approximately twenty-five *N. ornata*. No *Oscillatoria* was present.

Culture No. 2.—Fresh spring water, about twenty-five *N. ornata* and no *Oscillatoria*.

Culture No. 3.—Water from the same vessel as No. 1, about twenty-five *N. ornata* and an abundance of *Oscillatoria*.

Culture No. 4.—Fresh spring water, about twenty-five *N. ornata*, and an abundance of *Oscillatoria*.

All of the four cultures were kept in square watch glasses and these placed in a larger glass dish so that the external conditions were the same.

The cysts are formed along the sides of the vessel or in the detritus on the bottom of the vessel. They are spheroidal in

TABLE I.

Date.	I.	II.	III.	IV.
May 5th.......	*N. ornata*	*N. ornata*	*N. ornata*	*N. ornata*
May 6th.......	*N. elegans*	*N. elegans*	*N. ornata*	*N. ornata*
May 7th.......	*N. elegans* (a few cysts)	*N. elegans* (no cysts)	*N. ornata* (no cysts)	*N. ornata* (no cysts)
May 8th.......	*N. elegans* (about ½ cysts)	*N. elegans* (a few cysts)	*N. ornata* (no cysts)	*N. ornata* (no cysts)
May 9th.......	All encysted	*N. elegans* (a few cysts)	*N. ornata* (no cysts)	*N. ornata* (no cysts)
May 10th......	All encysted	*N. elegans* (about ½ cysts)	*N. ornata* (no cysts)	*N. ornata* (no cysts)
May 11th......	All encysted	All encysted	*N. ornata* (no cysts)	*N. ornata* (no cysts)

shape, brown and the wall is rather thick, presenting a scalloped appearance. They are a great deal smaller than the normal animals and in a newly formed cyst a light spot, the nucleus can be seen.

Excystment can be brought about by the addition of fresh water and *Oscillatoria*. After excystment the animal has the characteristics of *N. elegans* until it encounters food. A few hours after feeding it again presents the characteristics of *N. ornata*.

4. Conjugation.

In old cultures low in food supply, in which because of their age and food scarcity, encystment might be expected, conjugation has been encountered. Conjugation has only been observed under such conditions and always occurring after the animals have undergone the change from *N. ornata* to *N. elegans*. Thus, these adverse conditions of the culture seem to play the greatest rôle in conjugation.

The animals approach each other and fuse end to end. The nuclear stages during conjugation have not yet been worked out, but observations of living specimens as well as prepared materials show that the nucleus is not broken down in the process. Calkins (26) states that in many of the protozoa which have been thought to possess a single nucleus, there are in reality both micro- and macro-nuclei. These two nuclei are so closely associated that the micro-nucleus is not visible until conjugation takes place. I am convinced that this is not the case in *Nassula*, for during conjugation of protozoa where there are both types of nuclei, the macro-nucleus breaks down and the micro-nucleus takes the active part. In *Nassula*, as stated above, there is no such process, the conspicuous nucleus, which according to Calkins statement would be the macro-nucleus, functioning as does a micro-nucleus during conjugation.

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Summary.

1. *Nassula ornata* and *Nassula elegans* represent not two species, but two metabolic stages of a single species.

2. The well nourished phase, known as *Nassula ornata*, does not encyst. Lack of food, first, converts *Nassula ornata* into *Nassula elegans*, and, in time, drives the forms as *Nassula elegans* into encystment.

3. Binary fission occurs more frequently in the poorly fed forms (*Nassula elegans*) than in the well-nourished forms (*Nassula ornata*).

4. There is but one nucleus in this ciliate, there being no differentiation of the nuclear complex into micro- and macronuclei.

5. Conjugation involving this single nucleus takes place in stocks that have aged.

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DESCRIPTION OF PLATE.

Right aspect of *Nassula* in the intermediate phase, *i.e.*, a *Nassula elegans* not yet sufficiently nourished to assume the larger, spheroidal contour of *Nassula ornata*. *PH*, pharynx; *NU*, nucleus; *CV*, contractile vacuole. $\times$ 250.